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Immunologic Abnormalities in Pathogen-Free Cats Experimentally Infected with Feline Immunodeficiency Virus

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Blood mononuclear cells from 47 cats experimentally infected with feline immunodeficiency virus (FIV) were examined by using monoclonal antibodies directed against feline CD4 and CD8 homologs, a pan-T-cell antigen, and cell surface immunoglobulin. Significant inversion of the $CD4^+/CD8^+$ T-cell ratio was observed only in cats that were infected for 18 months or more. This inversion was associated with a decrease in the absolute numbers of $CD4^+$ T cells and a concomitant increase in $CD8^+$ cells. However, the total numbers of circulating T and B cells were not significantly reduced. Cats infected with FIV for 24 to 28 months also had significantly elevated levels of serum immunoglobulin G (IgG), but normal levels of IgA and IgM. The long-term decline in $CD4^+$ T cells and hypergammaglobulinemia observed in FIV-infected cats resemble the abnormalities occurring in humans after human immunodeficiency virus infection.

Feline immunodeficiency virus (FIV) is a T-lymphotropic lentivirus that has been associated with an acquired immunodeficiency syndrome-like condition of domestic cats (14, 16, 18, 20). Although much is known about the molecular properties, epidemiology, pathogenesis, and in vitro lymphotropism of FIV infection (7, 8, 14, 16, 18–20), little information has been presented on the potential of this virus to induce human immunodeficiency virus (HIV)-like immunologic abnormalities. Immunologic abnormalities that are common in HIV-infected people include a decline in the absolute numbers of $CD4^+$ lymphocytes beginning months or years after infection and a generalized B-cell abnormality characterized by hypergammaglobulinemia (for a recent review, see reference 17). We have thus undertaken the characterization of the immunologic alterations brought about by FIV infection of domestic, specific-pathogen-free cats kept in pathogen-free quarters, utilizing a panel of monoclonal antibodies to the feline CD4 (2) and CD8 (10) homologs, a pan-T-cell marker (1), and cell surface immunoglobulins (11). Serum levels of immunoglobulin G (IgG), IgM, and IgA were also measured. The results of these studies indicate that FIV infection, like its human counterpart, leads to a selective and delayed decline in the absolute levels of $CD4^+$ T cells, an eventual inversion of the $CD4^+/CD8^+$ T-cell ratio, and hypergammaglobulinemia.

Specific-pathogen-free domestic cats, obtained from the breeding colony of the Feline Retrovirus Research Laboratory and Nutrition and Pet Care Center, University of California, Davis, were housed in facilities of the Animal Resource Services at the same institution.

Infections were initiated by single or multiple intraperitoneal inoculations (1 ml per injection per cat) of FIV-infected blood or cell culture fluid (cell free). The cell culture supernatants were derived from primary T-lymphoblastoid cell cultures infected by blood mononuclear cells from kittens 2428 and 2429. The reverse transcriptase activity of primary T-lymphoblastoid culture fluid ranged from 1.25×10^5 to 2.5×10^5 cpm/ml.

Forty-seven cats were assigned to one of two groups. The first, consisting of 14 cats, served as uninfected specific-pathogen-free controls, and the second group, consisting of 33 cats, were examined at different times after FIV infection. The cats in both groups were of random sex and between 7 and 48 weeks old at the time of FIV inoculation.

Total leukocytes and erythrocytes were estimated by electronic counts. Platelet counts were done with a hemacytometer, and differential leukocyte counts were performed by microscopic evaluation of Wright-Leishman-stained blood smears.

Mouse monoclonal antibodies to feline CD4 and CD8 and pan-T-cell markers (Fel 7, FT2, and f43, respectively [1, 2, 11]) were used to evaluate mononuclear blood cells isolated from heparinized blood samples (5 ml) by Ficoll-Hypaque gradient centrifugation. The mononuclear blood cells were screened for CD4 and CD8 cell surface expression by two-color immunofluorescence flow cytometry (FACScan; Becton-Dickinson, Mountain View, Calif.) as previously described (5). The number of T cells was estimated by determining the absolute number of lymphocytes and the percentage of monoclonal antibody f43-reactive lymphoid cells. B-cell enumeration involved the use of IgM- and light chain-specific monoclonal antibodies CC4 and EA2, respectively (11). The concentrations of serum IgA and IgM were measured by an enzyme-linked immunosorbent assay, using the heterologous anti-α, anti-μ (CC4), and anti-light chain (EA2) antibodies (9). Serum IgG levels were estimated by radial immunodiffusion (Binding Site, San Diego, Calif. [13]).

The uninfected, control group of cats remained healthy throughout the course of this study. Cats infected with FIV often developed transient neutropenia 4 to 5 weeks after inoculation, which was accompanied with mild to moderate fever. The leukocyte and neutrophil counts returned to

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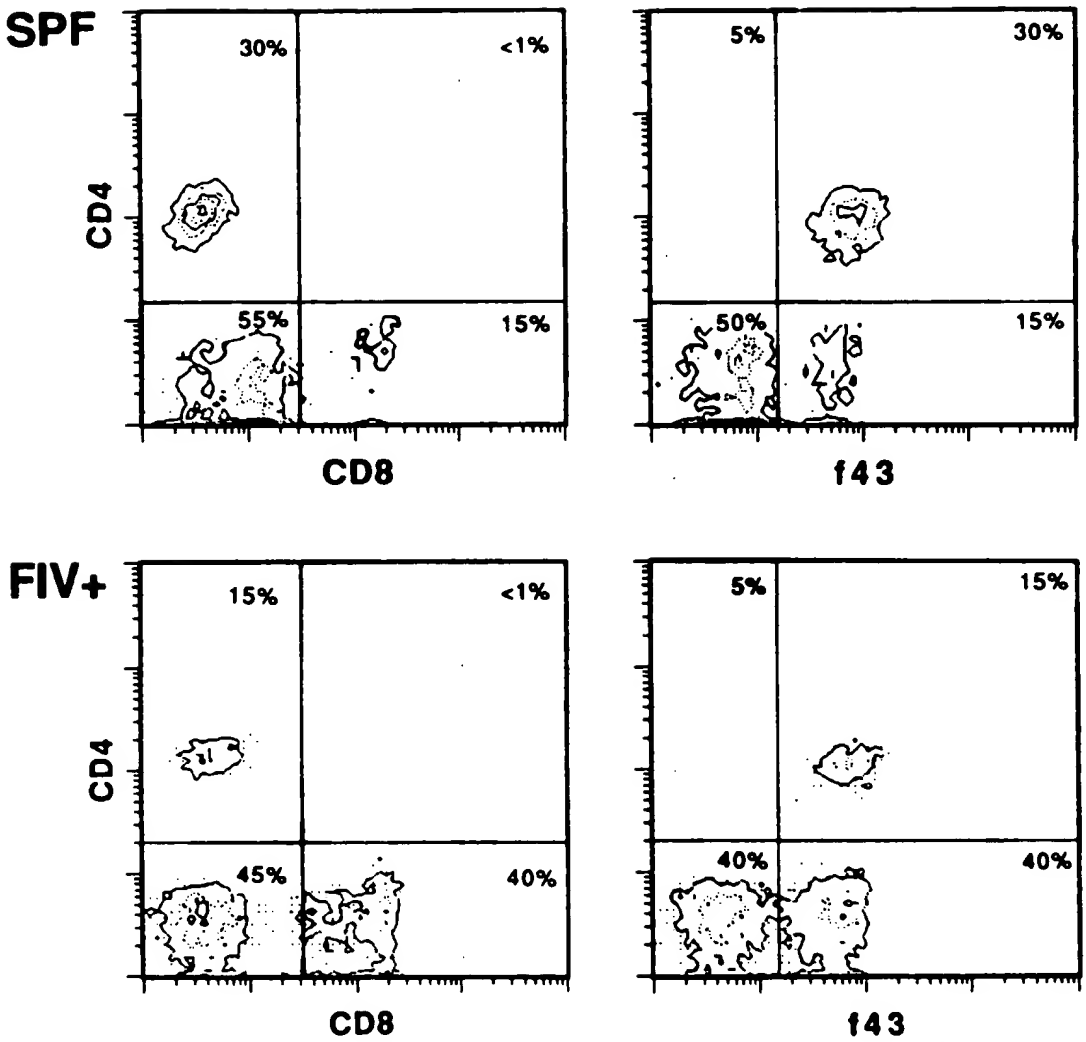

FIG. 1. Two-color immunofluorescence analysis of T-cell subpopulations in a control specific-pathogen-free (SPF) cat (top panels), and a cat infected with FIV for 24 months (bottom panels). Monoclonal antibodies reactive to the feline CD4 (Fel 7) and CD8 FT2 homologs as well as a pan-T-cell (f43) marker were employed for the analysis. Viable cells were initially stained with the CD8 or f43 mouse monoclonal antibodies followed by fluorescein isothiocyanate-conjugated goat antibodies to mouse IgG, and then with biotin-conjugated CD4 (Fel 7) antibodies and streptavidin phycoerythrin. Cellular immunofluorescence was evaluated by automated flow cytometry. Note the reduction in CD4 (Fel 7^+) T cells and the concomitant increase in CD8 T cells in the FIV-infected cat.

normal or near-normal levels after 13 weeks, as noted previously (20). Overt clinical immunodeficiency was not manifested during this study (2 years) after FIV infection.

The CD4 and CD8 subsets of T lymphocytes and their relative frequencies were examined by flow immunocytometry in specific-pathogen-free cats experimentally infected with FIV (Fig. 1), and absolute lymphocyte counts were performed on the same blood samples. Two-sample t test analysis of the T-cell subset values in the 47 test animals revealed no statistical differences between the mean $CD4^+/CD8^+$ cell ratio of noninfected cats (1.48 ± 0.45 [mean $\pm$ 1 standard deviation]) and those cats infected with FIV for 1.5 (1.4 ± 0.45), 7 (0.95 ± 0.33), and 12 (1.34 ± 0.45) months ($P \geq 0.05$ at each sample interval). The $CD4^+/CD8^+$ T-lymphocyte ratios for cats 18 and 24 months after infection were significantly altered (0.84 ± 0.10, $P \leq 0.005$ and 0.43 ± 0.17, $P \leq 0.005$, respectively) from those of the controls. However, the absolute numbers of T lymphocytes were not significantly different ($P \geq 0.05$) between control and FIV-infected cats over the first 2 years of infection. The alterations in circulating T-cell subsets were characterized by a decrease in both the percentage and absolute numbers of $CD4^+$ T cells ($P \leq 0.0005$ at 18 and 24 months), with a concomitant increase in the percentage and absolute numbers ($P \leq 0.05$ at 24 months) of $CD8^+$ T cells compared with uninfected control animals.

B lymphocytes in the circulation were enumerated by using monoclonal antibodies directed against feline IgM heavy and light chains. The percentage and absolute number of B lymphocytes were not significantly altered by FIV infection (Fig. 2). To examine the possibility that B lymphocytes in FIV-infected cats were undergoing polyclonal activation (12), serum levels of IgM, IgG, and IgA were measured. No significant alterations were observed in serum

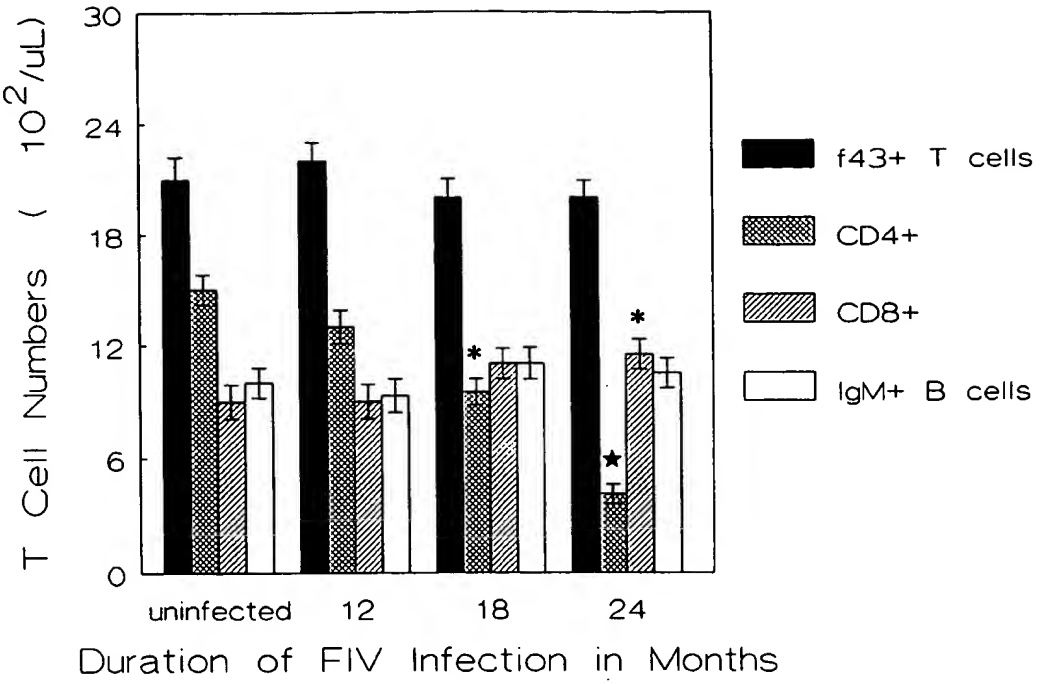

FIG. 2. Enumeration of T and B cells in uninfected and FIV-infected cats monitored over a 28-month period. The numbers of lymphocytes were determined by total and differential leukocyte counts, and the percentages of lymphocytes expressing f43, CD4, CD8, or IgM were determined by immunofluorescence. Bars indicate 1 standard error. ★, $P \leq 0.005$; *, $P \leq 0.05$.

IgM and IgA levels over the first 2 years of FIV infection (Table 1). However, the level of serum IgG was significantly increased ($P \leq 0.05$) in cats infected with FIV for 2 years or more.

The results of this study suggest that FIV infection alone has its primary effects on the immune systems of domestic cats. The immunologic abnormalities were not evident until 18 months or more after infection. The most significant immunologic abnormality observed in long-term, FIV-infected cats was a decrease in the absolute numbers of T cells bearing the feline homolog of the human CD4 cell surface antigen and an increase in the numbers of T cells possessing the CD8 cell surface antigen. Although changes were apparent in the relative proportions and numbers of the major T-cell subpopulations, the total numbers of the peripheral blood mononuclear cells bearing the feline pan-T-cell surface antigen were unaffected. The net effect of changes in the relative numbers of $CD4^+$ and $CD8^+$ T cells was an inversion of the $CD4^+/CD8^+$ T-cell ratio. The types of T-cell abnormalities seen in FIV-infected cats and the long latency between infection and the appearance of these abnormalities were identical to those described for HIV infection of humans (6).

TABLE 1. Serum immunoglobulin levels in FIV-infected and control cats[a]

Group (no. of cats)	Serum antibody concn (mg/ml [mean ± 1 SE])		
	IgG	IgM	IgA
Uninfected (7)	15.1 ± 6.6	0.90 ± .17	1.5 ± 0.10
FIV-infected (7)	32.6 ± 11.1[b]	0.90 ± .07	1.7 ± 0.10

[a] Serum IgG, IgM, and IgA levels were measured in FIV-infected cats 24 to 28 months postinfection and in noninfected age-matched controls. The levels of serum IgM and IgA were determined by quantitative enzyme-linked immunosorbent assay, using isotype-specific monoclonal antibodies. Serum IgG levels were determined by radial immunodiffusion, using a commercial kit.

[b] Significantly different ($P \leq 0.005$) compared with age-matched uninfected cats.

The B-cell systems of FIV-infected cats also appeared to be affected by FIV infection. Although the total numbers of B cells in the peripheral blood were normal, a twofold increase in the levels of serum IgG was seen in cats that were infected for longer than 24 to 28 months. B-cell abnormalities in HIV-infected people often result in decreased antibody responses to many non-HIV antigens but an overall increase in the total levels of serum immunoglobulins (4, 12). The excess immunoglobulins apparently reflect the response to HIV itself and not to unrelated antigens (3). Whether such a phenomenon applies to the hypergammaglobulinemia of FIV-infected cats remains to be determined.

Although many of the FIV-infected cats acquired pronounced immunologic abnormalities during the course of this study, all of them were outwardly healthy. This was not unexpected, because the cats were specific-pathogen-free at the time of FIV infection and were kept in pathogen-free quarters all of their lives. It would be of interest to see whether these cats could handle common feline pathogens in a normal manner. Recent studies have shown specific-pathogen-free FIV-infected cats to be much more sensitive to the primary disease induced by a common opportunistic-type rickettsial pathogen of cats, *Haemobartonella felis* (J. George and N. C. Pedersen, University of California, Davis, unpublished observations).

The relatively long period between initial FIV infection and the appearance of immunologic abnormalities makes FIV infection of cats an attractive animal model for studies of disease-potentiating cofactors of human HIV infection.

Indeed, cats that are asymptomatically infected with feline leukemia virus, another common retrovirus of cats, develop a much more severe form of FIV after experimental infection with the latter virus (15). Instead of taking 18 months or more to demonstrate declines in $CD4^+$ T-cell levels, decreases occurred in 8 months or less in dually infected cats.

It remains to be determined whether the mechanisms behind the $CD4^+$ T-cell depletion seen in FIV-infected cats and HIV-infected people are similar. Even though our comparative knowledge of FIV infection of cats is still incomplete, this and other published data support the idea that FIV-induced immunodeficiency syndrome may serve as a useful small animal model of acquired immunodeficiency syndrome.

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